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Salt-tolerant *Pseudomonas extremorientalis* Able to Stimulate Growth of *Silybum marianum* under Salt Stress

Dilfuza Egamberdieva[1*] • Dilfuza Jabborova[1] • Nilufar Mamadalieva[2]

[1] Department of Biotechnology and Microbiology, Faculty of Biology and Soil Sciences, National University of Uzbekistan, 100174 Tashkent, Uzbekistan
[2] Laboratory of the Chemistry of Glycosides, Institute of the Chemistry of Plant Substances AS RUz, Mirzo Ulugbek Str. 77, Tashkent 100170, Uzbekistan
Corresponding author: * egamberdieva@yahoo.com

ABSTRACT

The effect of root-colonizing *Pseudomonas extremorientalis* TSAU20 on the root and shoot growth and biomass was determined in the medicinal plant *Silybum marianum* (milk thistle) under salinated conditions. Four salinity levels (25, 50, 75 and 100 mM) were maintained in a gnotobiotic system using NaCl salt. Shoot and root growth were reduced as much as 42% and fresh weight as much as 31% at 100 mM NaCl. Inoculation of salt-stressed milk thistle with *P. extremorientalis* TSAU20 significantly improved root length (90%), shoot length (66%) and total fresh weight (64%) at 100 mM NaCl compared to control plants. *P. extremorientalis* TSAU20 also increased the root and shoot length and dry weight of milk thistle in non-saline (0.8 dS m^{-1}), slightly saline (E_C 2.3 dS m^{-1}) and saline (E_C 7.1 dS m^{-1}) soil. The root length increased by 49%, shoot length by 67% and dry weight by 21%. The strain was able to survive in the rhizosphere of plants. The results presented here make it possible for recommending root-colonizing, auxin-producing *P. extremorientalis* TSAU20 to alleviate salt stress of milk thistle grown under conditions of soil salinity.

Keywords: medicinal plant, plant growth stimulation, rhizosphere bacteria, salinity

INTRODUCTION

Milk thistle (*Silybum marianum*) has been used for more than 2000 years as a natural remedy for treating hepatitis, gastrointestinal disorders and especially for its protective effects on the liver (Ramasamy and Agarwal 2008). Agarwal *et al.* (2006) showed that a silymarin flavonolignan from milk thistle has anti-inflammatory, anti-oxidant as well as anti-metastatic activity by modulating specific proteins. The seeds of milk thistle contain silymarin, silybinin, silychristin, silydianin, phospholipids and a high content of vitamin E (Hadolin *et al.* 2001), which are useful against liver diseases and help maintain healthy liver function (Henywood and Harborne 1977; Tedesco *et al*. 2004). In addition, milk thistle fruits and their extracts are used as ingredients of animal feed mixtures and functional foods (Hadolin *et al*. 2001; Urbanczyk *et al*. 2002). Due to a growing demand for silymarin by the pharmaceutical industry, milk thistle is commercially cultivated in Egypt, China, Iran, Pakistan, Uzbekistan, Canada, Poland and other European countries (Sadaqat *et al*. 1983; Anonymous 1995; Andrzejewska and Sadowska 2008).

Field-scale trials of milk thistle in Saskatchewan, Canada yielded up to 378 kg/ha with mechanical harvesting. This is about 1/3 to 1/4 of the yield reported in Europe and Africa, respectively where growing seasons are longer (Barl *et al.* 1996). In western Rajasthan, India, the total area for milk thistle cultivation is about 200 ha (500 acres), in Panjin, China more than 2000 ha, and in Poland 1500 ha, ensuring that enough sources of raw materials for deriving silymarin (Lozykowska 2009). In Slovakia, the highest yield of milk thistle fruits recorded was 1832.0 kg/ha (Haban *et al.* 2009).

Successful cultivation of milk thistle depends on biotic and abiotic factors. Several species of pest insects feed on milk thistle and reduce plant yield (Rumicska 1991; Abdel-Moniem 2002; Andrzejewska *et al*. 2006). The widespread application of agrochemicals to intensify crop cultivation and extensive flood irrigation with poor quality water causes soil salinity which causes a significant reduction in the yield of medicinal plants (Munns 2003; Ashraf 2004), including milk thistle. It is important to reduce the use of chemical fertilizers and pesticides for cultivation of medicinal plants since they are typically consumed without being further processed after harvest (Banchio *et al*. 2008). One of the possible measures to improve plant growth in such conditions is to use plant growth-promoting bacterial inoculants which can prevent the deleterious effects of stressors caused by the environment (Lugtenberg and Kamilova 2004). In previous studies, plant growth-promoting bacteria (PGPR) improved the growth of tomato, pepper, wheat and bean under salinated conditions (Mayak *et al*. 2004; Yildirim and Taylor 2005; Egamberdieva and Kucharova 2009). Although there are several reports on the positive effects of PGPR on the growth of crops under ecologically stressed conditions, few studies have been conducted on the effects of inoculation with PGPR on the growth and development of medicinal plants. The purpose of the present study was to evaluate if inoculation of milk thistle (*Silybum marianum*) with *Pseudomonas extremorientalis* could enhance salt tolerance and plant growth under saline soil conditions.

MATERIALS AND METHODS

Plant and bacteria

Milk thistle (*Silybum marianum* L. Gaertn.) was obtained from the Tashkent University of Agriculture. The bacterial strain *P. extremorientalis* TSAU20 was obtained from the culture collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan (Egamberdieva and Kucharova 2009). This strain was isolated from the rhizosphere of wheat (*Triticum aestivum* cv. 'Turon') grown in salinated Uzbek soil.

The strain produces IAA, protease and is able to grow in the presence of high salt concentrations, as much as 5%. Stock culture was stored at –80°C in 20% glycerol, and, before being used, they were grown overnight at 28°C and 120 rpm in King's medium B (KB) (King *et al*. 1954) or on KB agar medium at 28°C for 24 h.

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Original Research Paper

Plant growth promotion in gnotobiotic systems and pots

The effects of inoculation with *P. extremorientalis* TSAU20 on the growth of milk thistle seedlings growing under salt stress was preliminary studied under gnotobiotic conditions. Experiments were carried out in test tubes (25 mm in diameter, 200 mm length) described by Simons *et al*. (1996), containing 60 g of sterilized and washed sand, and soaked with 10% Plant Nutrient Solution (PNS) consisting of 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4 (Kuiper *et al*. 2001) and micronutrients (KI 0.75 mg l^{-1}, H_3BO_3 3 mg l^{-1}, $MnSO_4 \cdot H_2O$ 10 mg l^{-1}, $ZnSO_4 \cdot 5H_2O$ 2.0 mg l^{-1}, $Na_2MoO_4 \cdot 2H_2O$ 0.25 mg l^{-1}, $CuSO_4 \cdot 5H_2O$ 0.025 mg l^{-1}, $CoCL_2 \cdot 6H_2O$ 0.025 mg l^{-1}, pH adjusted to 5.8) (Hoffland *et al*. 1989). Salinity conditions were established by adding 25, 50, 75 and 100 mM NaCl into PNS. For seed inoculation, *P. extreme orientalis* TSAU20 was grown overnight in KB broth (King *et al*. 1954) at 28°C. One ml of bacterial culture was pelleted by centrifugation and the supernatant was discarded. Cell pellets were washed with 1 ml of phosphate-buffered saline (PBS, 20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and suspended in PBS. The cell suspension was diluted to an optical density of 0.1 at 620 nm (Hitachi U-1800 Spectrophotometer, Hitachi Ltd., Tokyo, Japan) corresponding to a cell density of 10^8 cells/ml. Seeds were sterilized for 5 min with concentrated sulphuric acid, followed by 70% ethanol for 3 min and rinsed five times with sterile, distilled water. Seed germination was carried out in 85 mm × 15 mm tight fitting plastic Petri dishes with 5 ml of water.

Germinated seeds were placed in the bacterial suspension with sterile forceps and were shaken gently for a few seconds. After standing for 10 min, inoculated seedlings were planted in sterile glass tubes, one seed/tube. Ten replicate tubes were used per treatment. The seedlings were grown in a growth cabinet with a 16-h light period at 22°C and an 8-h dark period at 16°C. After 14 days of growth the length of shoots and roots, and fresh weight of whole plants were measured.

The effect of *P. extremeorientalis* TSAU20 on the growth of milk thistle was also measured in plastic pots containing 300 g of the non-saline (0.8 dS m^{-1}), slightly saline (E_C 2.3 dS m^{-1}) and saline (E_C 7.1 dS m^{-1}) soil mentioned above. The milk thistle seeds were sterilized, allowed to germinate and inoculated with bacteria, as described above. The inoculation treatments were set-up in a randomised design with 10 replications. The plants were grown under open natural conditions and temperatures ranged between 24 and 26°C during the day and between 10 and 12°C at night. After six weeks of growth the root and shoot length and dry weight of the whole plants was determined.

Root colonization assay

For the root tip colonization assay, seedlings were inoculated with bacterial strains and grown under gnotobiotic conditions for 14 days, as described above. To isolate bacteria from the rhizosphere, the complete sand column was carefully removed from the tube. Most of the still adhering rhizosphere sand was removed and a length of 1 cm root tip was cut off with caution to prevent cross contamination from upper root parts. Bacterial cells were removed from the root tip by vortexing in 1 ml PBS. The homogenates were serially diluted and were spread on agar plates and plated on KB.

Survival of bacterial strains

A spontaneous and stable rifampicin (200 µg/ml)-resistant *P. extremorientalis* TSAU 20 mutant was used to investigate bacterial survival in the root of milk thistle grown in saline soil. Milk thistle seedlings were coated with bacteria by dipping them in bacterial suspension as described above and grown in plastic pots (9 cm diameter; 12 cm deep) containing 350 g of soil. Plants were grown at 20-22°C during the day and at 14-16°C at night. After 6 weeks, plants were harvested and the adhering soil was removed from milk thistle roots and 1 g of roots was shaken in 9 ml of sterile PBS. The resulting suspensions were evaluated for colony-forming units (cfu) according to the dilution-plate method in KB agar following the addition of 200 µg/ml rifampicin. After incubation for 2-3 days at 28°C, the reisolated, rifampicin-resistant strains were identified for their colony characteristics (Höflich *et al*. 1995).

Seedling response to IAA

Seeds of milk thistle were sterilized as described above and seed germination was carried out in 85 mm × 15 mm tight fitting plastic Petri dishes with 5 ml of test solution consisting of no (control) or 100 mM NaCl. Filter paper (Whatman No. 2) was soaked in a solution of 1, 0.1, 0.01, 0.001 µM IAA. Twenty uniform seeds were spread in each Petri dish with three replications. The milk thistle seeds were also inoculated with *P. extremorientalis* TSAU20 which produces IAA (5.7 µg/ml) (Egamberdieva and Kucharova 2009) and were aseptically placed in a Petri dish moistened with water and with 100 mM NaCl solution. All seed were germinated in a plant growth chamber at 28°C. The percent germination was recorded after 5 days. The root and shoot lengths of the germinated seeds which exceeded 0.5 mm in length were measured and recorded.

Statistical analysis

Analysis of variance was performed using Excel version 11 for Windows 2007 (Microsoft Corp.), and least significant differences (LSD) were applied to compare means at $P < 0.05$. Standard error and LSD were calculated.

RESULTS

The growth-promoting effect of *P. extremorientalis* TSAU20 strain was preliminarily studied by growing inoculated salt-stressed milk thistle seedlings for 14 days in a gnotobiotic sand system. The shoot and root length of milk thistle was reduced as much as 42% and fresh weight as much as 31% in 100 mM NaCl. Inoculation of salt-stressed (100 mM NaCl) milk thistle with *P. extremorientalis* TSAU20 significantly stimulated root (90%), shoot length (66%) and fresh weight (64%) compared to control plants (**Table 1**).

The root-tip colonization of *P. extremorientalis* TSAU20 was slightly reduced as salinity increased. The bacterial colonization in the rhizosphere was 15.6 (10^3 cfu/cm root tip) at 0 mM NaCl and 9.2 (10^3 cfu/cm root tip) at 100 mM NaCl (**Table 1**).

The inoculation of milk thistle with *P. extremorientalis* TSAU20 significantly increased root and shoot length and dry weight of plants in non-saline (E_C 0.8 dS m^{-1}), weak (E_C 2.3 dS m^{-1}) and saline (E_C 7.1 dS m^{-1}) soils by as much as 50% (**Figs. 1, 2**). Root length increased by 49%, shoot

Table 1 The length of roots, shoots and fresh weight of whole plants of milk thistle when seedlings were inoculated with *P. extremorientalis* strain TSAU20. Plants were grown in the gnotobiotic sand system under salt stress for two weeks.

Salinity	Control			*P. extremorientalis* TSAU20			
	Shoot	Root	Fresh weight	Shoot	Root	Fresh weight	10^3 CFU/ cm root tip
0 mM	3.6 ± 0.5	14.7 ± 1.7	0.53 ± 0.09	4.5 ± 0.6	18.6* ± 0.5	0.69 ± 0.11	15.6 ± 2.5
25 mM	3.2 ± 0.5	12.6 ± 1.8	0.47 ± 0.07	4.0 ± 0.8	18.0* ± 1.0	0.67* ± 0.12	13.5 ± 1.0
50 mM	3.0 ± 0.7	11.7 ± 1.7	0.46 ± 0.08	4.3* ± 0.5	19.2* ± 1.0	0.68* ± 0.12	14.4 ± 1.6
75 mM	3.1 ± 0.3	11.0 ± 0.8	0.44 ± 0.06	4.5 ± 1.3	19.5* ± 1.3	0.66* ± 0.11	9.8 ± 2.1
100 mM	2.1 ± 0.6	10.5 ± 1.3	0.37 ± 0.11	4.0* ± 0.8	17.5* ± 1.3	0.61* ± 0.04	9.2 ± 1.8

* Significantly different from the negative control at $P < 0.05$

Table 2 The effect of *P. extremorientalis* TSAU20 and different concentrations, 0, 0.1, 0.01, and 0.001 µM of auxin (IAA) on the shoot and root growth of milk thistle seedling in non saline and saline (100 mM NaCl) conditions.

Treatments	0 mM NaCl		100 mM NaCl	
	Root[a]	Shoot[a]	Root[a]	Shoot[a]
Control	2.5 ± 0.5	3.0 ± 1,0	1.75 ± 0.25	2.5 ± 0.5
TSAU20	4.2* ± 0.3	4.0 ± 1.0	3.3* ± 0.6	3.5* ± 0.5
IAA[b] 1.0	3.3 ± 0.6	3.7 ± 0.6	1.8 ± 0.3	3.2 ± 0.7
0.1	3.7 ± 1.5	4.7 ± 1.1	4.0* ± 1.0	4.7* ± 0.6
0.01	5.3* ± 0.6	4.7 ± 0.6	3.7* ± 0.6	4.6* ± 0.5
0.001	5.0* ± 1.0	5.2* ± 0.3	4.0* ± 1.4	4.5* ± 0.7

[a] root/shoot length, [b] µM, * significantly different $p<0.05$

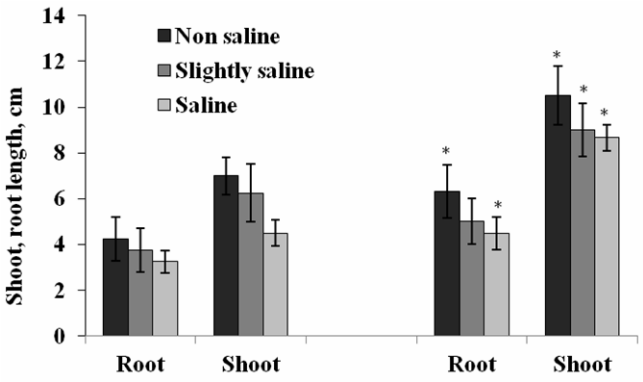

Fig. 1 The length of roots/shoots of milk thistle grown under non-saline (0.8 dS m^{-1}), slightly saline (E_C 2.3 dS m^{-1}) and saline (E_C 7.1 dS m^{-1}) soil and inoculated with *P. extremorientalis* strain TSAU20. Plants were grown in a greenhouse for six weeks. Values represent means for 10 plants (N = 10), with error bars showing standard deviation. Columns marked with an asterisk differed significantly from control plants not inoculated with bacteria at $P < 0.05$.

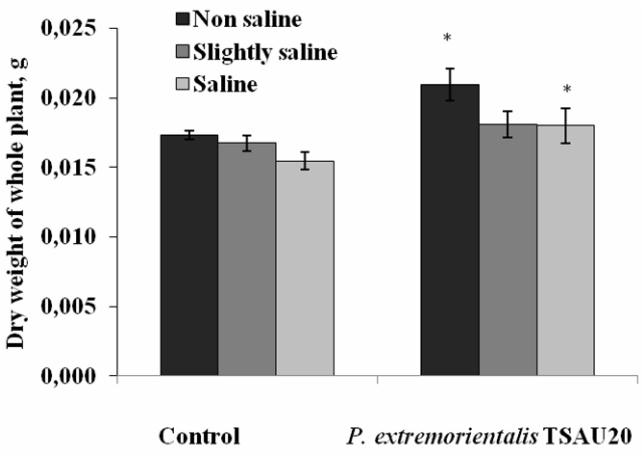

Fig. 2 The dry weight of whole plants grown under non-saline (0.8 dS m^{-1}), slightly saline (E_C 2.3 dS m^{-1}) and saline (E_C 7.1 dS m^{-1}) soil and inoculated with *P. extremorientalis* strain TSAU20. Plants were grown in a greenhouse for six weeks. Values represent means for 10 plants (N = 10), with error bars showing standard deviation. Columns marked with an asterisk differed significantly from control plants not inoculated with bacteria at $P < 0.05$.

length by 67% and dry weight by 21%.

The rifampicin-resistant mutant of *P. extremorientalis* TSAU20 was able to survive in the root of six-weeks-old milk thistle grown in non-saline, weak and saline soils. However, bacterial colonization decreased from 10.8 ± 2.1 (10^3 cfu/g root, non-saline soil) to 6.8 ± 1.6 (10^3 cfu/g root).

The application of IAA reversed the growth-inhibiting effect of salt stress in milk thistle at a low concentration. Considering the length of root to be 2.5 cm, and that of the shoot to be 3.0 cm (control, no salt), 100% of data shows that salt stress at 100 mM reduced root length to 1.7 cm (30%) and shoot length to 2.5 cm (by 16.7%). The application of IAA reversed the growth-inhibiting effect of salt-stress at 0.1, 0.01 and 0.001 µM to a certain extent in both shoots and roots (**Table 2**). The higher concentration of IAA (1.0 µM) had no effect on the stimulation of both shoot and root length. Only low concentrations of IAA (0.01, 0.001 µM) stimulated root and shoot length of milk thistle seedlings in non-saline and saline conditions. The inoculation of milk thistle seedling with IAA-producing *P. extremorientalis* TSAU20 strain significantly increased seedling root length up to 90% and shoot length up to 46% at 100 mM NaCl compared to control plants (**Table 2**).

DISCUSSION

Soil salinity reduces plant growth due to the toxic effects of NaCl, to the ability of the root system to control entry of ions to the shoot and to slowing down water uptake of plants (Hajibagheri *et al*. 1989). High salinity causes severe damage to medicinal plants, including growth inhibition, impaired metabolism, loss of production and quality (Ashraf 2004).

In our study, inoculation of unstressed and salt-stressed milk thistle with *P. extremorientalis* TSAU20 significantly improved root and shoot growth of plants. Similar observations were reported by other authors in which *Pseudomonas fluorescens* stimulated growth and yield of Madagascar periwinkle (*Catharanthus roseus*) under drought stress (Attia and Saad 2001; Jaleel *et al.* 2007). Karthikeyan *et al*. (2010) reported that PGPR strains *Pseudomonas* and *Bacillus* significantly increased plant height, root length, root girth and alkaloid content in *C. roseus* relative to the control.

The colonization and survival of the introduced bacterial strain in the highly competitive rhizosphere environment is important for successful inoculation (Wessendorf and Lingens 1989; Lugtenberg *et al*. 1999). We observed that colonization of *P. extremorientalis* TSAU20 was slightly decreased at 100 mM NaCl. Similar results were reported by Paul and Nair (2008) although the root colonization potential of the salt-tolerant *Pseudomonas* strain was not hampered by higher salinity. This strain was able to tolerate salt stress up to 4% NaCl (Egamberdieva and Kucharova 2009). In our study the rifampicin-resistant *P. extremorientalis* TSAU20 mutant survived in the rhizosphere of milk thistle due to its competitiveness and persistence in saline soil.

We tried to evaluate on which mechanisms the observed alleviation of salt stress and stimulation of growth milk thistle can be based. Mechanisms by which bacteria are able to alleviate salt stress in plants and promote plant growth include mobilization of nutrients (Lugtenberg *et al*. 2001; Lifshitz *et al*. 2004), production of ACC deaminase, which can lower the level of the plant stress hormone ethylene (Glick *et al*. 1998, 2007; Belimov *et al*. 2009) and production of phytohormones (Costacurta and Vanderleyden 1995; Spaepen *et al*. 2009). The bacterial strain *P. extremorientalis* TSAU20 negatively impacted ACC-deaminase and produced IAA in high saline conditions (up to 3% NaCl) (Egamberdieva and Kucharova 2009). It was previously reported that salt stress reduces the supply of phytohormones, for exmaple from roots to shoots (Itai *et al*. 1968; Sakha-

butdinova *et al*. 2003). In this condition, application of exogenous phytohormones such as gibberellins or auxins produced some benefit in alleviating the adverse effects of salt stress and they also improved germination and seedling growth in salinated conditions (Khan *et al*. 2004; Afzal *et al*. 2005). We also observed that the application of IAA reversed the growth-inhibiting effect of salt-stress in milk thistle at a low concentration. It has also been suggested that root-colonizing bacteria that produce phytohormones, when bound to the seed coat of a developing seedling, may act as a mechanism for plant growth stimulation and that these organisms can prevent the deleterious effects of stress by the environment (Frankenberger and Arshad 1995). Therefore, it is likely that by colonizing the roots, *P. extremorientalis* TSAU20 produced IAA, alleviating salt stress and stimulating milk thistle growth.

The results presented here make it possible to recommend root-colonizing, auxin-producing *P. extremorientalis* TSAU20 to alleviate salt stress of milk thistle grown under saline soil.

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REFERENCES

Abdel-Moniem ASH (2002) The seed-head weevil, *Larinus latus* herbst (*Coleoptera*: *Curculionidae*) as a new record in Egypt on the milk thistle, *Silybum marianum* (L.) (*Asteraceae: Compositaea*). *Archives of Phytopathology and Plant Protection* **35**, 157-60

Afzal I, Basra Sh, Iqbal A (2005) The effect pf seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *Journal of Stress Physiology and Biochemistry* **1**, 6-14

Agarwal R, Agarwal C, Ichikawa H, Singh RP, Aggarwal BB (2006) Anticancer potential of silymarin: From bench to bed side. *Anticancer Research* **26**, 4457-4498

Andrzejewska J, Sadowska K (2008) Effect of cultivation conditions on the variability and interrelation of yield and raw material quality in milk thistle (*Silybum marianum* (L.) Gaertn.). *Acta Scientiarum Polonorum – Agricultura* **3**, 3-12

Andrzejewska J, Lamparski R, Skinder Z (2006) Assessing the extent of damage caused by *Cleonus piger* Scop. and other entomofauna in the cultivation of milk thistle *Silybum*. *Journal of Plant Protection Research* **1**, 49-59

Anonymous (1995) Market report. *Herbalgram* **39**, 68

Ashraf M (2004) Photosynthetic capacity and ion accumulation in a medicinal plant henbane (*Hyoscyamus niger* L.) under salt stress. *Journal of Applied Botany* **78**, 91-96

Attia FA, Saad OA (2001) Biofertilizers as potential alternative of chemical fertilizer for *Catharanthus roseus* G. Don. *Journal of Agricultural Science* **26 (11)**, 7208-7193

Banchio E, Bogino P, Zygadlo J, Giordano W (2008) Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. *Biochemical Systematics and Ecology* **36 (10)**, 766-771

Barl B, Loewen D, Svendsen E (1996) Saskatchewan Herb Database. Department of Horticulture Science, University of Saskatchewan, Saskatoon, SK

Belimov AA, Dodd IC, Hontzeas N, Theobaldm J, Safronova VI, Davies WJ (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytologist* **181**, 435-447

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. *Critical Reviews in Microbiology* **21**, 1-18

Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biology and Fertility of Soils* **45**, 561-573

Frankenberger JWT, Arshad M (1995) Microbial synthesis of auxins. In: *Phytohormones in Soils*, Marcel Dekker Inc., New York, pp 35-71

Glick BR, Penrose DM, Li JP (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *The Journal of Theoretical Biology* **190**, 63-68

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. *Plant Pathology* **119**, 329-339

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biology and Biochemistry* **37**, 395-412

Haban M, Otepka P, Kobida L, Habanova M (2009) Production and quality of milk thistle (*Silybum marianum* [L.] Gaertn.) cultivated in cultural conditions of warm agri-climatic macroregion. *Horticultural Science* **36 (2)**, 25-30

Hadolin M, Skerget M, Knez Z, Bauman D (2001) High pressure extraction of vitamin E-rich oil from *Silybum marianum*. *Food Chemistry* **74**, 355-364

Hajibagheri MA, Yeo AR, Flowers TJ, Collins JC (1989) Salinity resistance in *Zea mays* fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipid. *Plant Cell and Environment* **12**, 753-757

Heywood VH, Harborne JB, Torner BL (1977) *The Biology and Chemistry of Compositae*, Acadamic Press, London, 414 pp

Hoffland E, Findenegg GR, Nelemans JA (1989) Solubilization of rock phosphate by rape. *Plant and Soil* **113**, 161-165

Höflich G, Wiehe W, Hecht-Buchholz CH (1995) Rhizosphere colonization of different growth promoting *Pseudomonas* and *Rhizobium* bacteria. *Microbiological Research* **150**, 139-147

Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R, Panneerselvam R (2007) *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. *Colloid and Surfaces* **60 (1)**, 7-11

Itai C, Richmond AE, Vaada Y (1968) The role of root cytokines during water and salinity stress. *Israel Journal of Botany* **17**, 187-195

Karthikeyan B, Joe MM, Jaleel CA, Deiveekasundaram M (2010) Effect of root inoculation with plant growth promoting rhizobacteria (PGPR) on plant growth, alkaloid content and nutrient control of *Catharanthus roseus* (L.) G. Don. *Natura Croatica* **1**, 205-212

Khan MA, Gul B, Darrell J (2004) Weber Action of plant growth regulators and salinity on seed germination of *Ceratoides lanata. Canadian Journal of Botany* **82**, 37-42

King EO, Ward MK, Raney DC (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**, 301-307

Kuiper I, Bloemberg GV, Lugtenberg BJ (2001) Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-(PAH)-degrading bacteria. *Molecular Plant-Microbe Interactions* **14**, 1197-1205

Lifshitz R, Kloepper JW, Kozlowski M, Simonson C, Carlson J, Tipping EM, Lugtenberg BJJ, Kamilova FD (2004) Rhizosphere management: Microbial manipulation for biocontrol. In: Goodman RM (Ed) *Encyclopedia of Plant and Crop Science*, Marcel Dekker Inc., New York, pp 1098-1101

Lozykowska KS (2009) Medicinal plant seeds as an element of increasing biodiversity of grassland. SALVERE - Regional Workshop in Poland, pp 45-47

Lugtenberg BJJ, Kravchenko LV, Simons M (1999) Tomato seed and root exudate sugars: Composition, utilization by *Pseudomonas* biocontrol strains and role in rhizosphere colonization. *Environmental Microbiology* **1**, 439-446

Lugtenberg BJJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomanas*. *Annual Review of Phytopathology* **39**, 461-490

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science* **166**, 525-530

Munns R (2003) Comparative physiology of salt and water stress. *Plant Cell and Environment* **25**, 239-250

Paul D, Nair S (2008) Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *Journal of Basic Microbiology* **48**, 1-7

Ramasamy K, Agarwal R (2008) Multitargeted therapy of cancer by silymarin. *Cancer Letters* **269**, 352-362

Rumicska A (1991) *Poradnik Plantatora Ziyi*, PWRiL, Poznac, pp 301-305

Sadaqat H, Sabir A, Sabir SA, Khan Aziz P (1983) Experimental cultivation of *Silybum marianum* and chemical composition of its oil. *Pakistan Journal of Scientific and Industrial Research* **26**, 244-246

Sakhabutdinova AR, Fatkhutdinova DR, Bezrukova MV, Shakirova FM (2003) Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulgarian Journal of Plant Physiology* **21**, 314-319

Simons M, van der Bij AJ, Brand I, De Weger LA, Wijffelman CA, Lugtenberg BJ (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Molecular Plant-Microbe Interactions* **9**, 600-607

Spaepen S, Vanderleyden J, Okon Y (2009) Plant growth-promoting actions of rhizobacteria. *Advances in Botanical Research* **51**, 283-320

Tedesco D, Tava A, Galletti S, Tameni M, Varisco G, Costa A, Steidler S (2004) Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. *Journal of Dairy Science* **87**, 2239-2247

Urbaczyk J, Hanczakowska E, Witkiewicz M (2002) Herb mixture as an antibiotic substitute in pig feeding. *Medycyna Weterynaryjna* **11**, 887-889

Wessendorf J, Lingens F (1989) Effect of culture and soil conditions on survival of *Pseudomonas fluorescens* R1 in soil. *Journal Applied Microbial Biotechnology* **31**, 97-102

Yildirim E, Taylor AG (2005) Effect of biological treatments on growth of bean plants under salt stress. *Annual report of the Bean Improvement Cooperative* **48**, 176-177

Zalesk I (1987) Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Canadian Journal of Microbiology* **33**, 390-395